

TRANSDUCTIONAL HETEROGENOTES IN *ESCHERICHIA COLI*¹

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THE transduction of the *Gal*⁺ factor (ability to ferment galactose) to *Gal*⁻ mutants of *Escherichia coli* K12 has been described in a previous report (MORSE, LEDERBERG, and LEDERBERG 1956). The galactose positive transduction clones were often found to be unstable and to throw off *Gal*⁻ types about once per thousand divisions. We postulated that the transformed cells were heterogenotic (heterozygous for the transduced fragment), and that the instability was a result of segregation. This process has been studied in more detail with several non-allelic *Gal*⁻ mutants. Since transduction genetics is a system analogous but not identical with sexual crossing, which also occurs in these strains, a distinctive terminology is a useful tool for integrating hypothesis and experiment. The following definitions are given for reference at this point. Their applications will be amplified in the experimental report.

GLOSSARY AND SYMBOLS

Genetic transduction—transfer of a genetic fragment from one cell to another.

Exogenote—a chromosome fragment; usually relates to the donor in transduction.

Endogenote—homologous part of the intact chromosome which corresponds to a given exogenote; usually relates to the recipient in transduction.

Syngenote—(cf. synkaryon) a cell whose genetic complement includes an exogenote (i.e. is hyperploid for a fragment).

Heterogenote—(cf. heterozygote) a syngenote in which the exogenote and endogenote differ in one or more markers.

Homogenote—(cf. homozygote) a syngenote in which the exogenote and endogenote carry the same marker.

Transduction clone—the entire vegetative progeny of a cell which has received an exogenote, including nonsyngenotic and syngenotic descendants.

Symbols: /_{ex} Syngenotes will be given as endogenote /_{ex} exogenote; —X Transduction will be symbolized as donor —X recipient.

CULTURES

The mutants that have been used in this study were accumulated in a variety of stocks, table 1. The *Gal*⁻ mutations, as will be shown, are determined at different

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TABLE 1
Description of principal cultures

Mutant ²	Type culture	Method of isolation of <i>Gal</i> marker	Genotype ¹
<i>Gal</i> ₁ ⁻	W-750	UV, EMB lactose	<i>F</i> ⁺ <i>Lp</i> ⁺ <i>M</i> ⁻ <i>Lac</i> ₁ ⁻ <i>V</i> ₁ ^r
<i>Gal</i> ₂ ⁻	W-902	UV, EMB galactose	<i>F</i> ⁻ <i>Lp</i> ⁺ <i>T</i> ⁻ <i>L</i> ⁻ <i>Th</i> ⁻ <i>Mal</i> ₁ ⁻³
<i>Gal</i> ₃ ⁻	W-892	spontaneous, EMB lactose	<i>F</i> ⁻ <i>Lp</i> ⁺ <i>T</i> ⁻ <i>L</i> ⁻ <i>Th</i> ⁻ <i>Lac</i> ₁ ⁻ <i>Mal</i> ₁ ⁻
<i>Gal</i> ₄ ⁻	W-518	UV, EMB lactose	<i>F</i> ⁺ <i>Lp</i> ⁺ <i>M</i> ⁻ <i>Lac</i> ₁ ⁻ <i>V</i> ₁ ^r
<i>Gal</i> ₅ ⁻	W-677	UV, EMB galactose	<i>F</i> ⁻ <i>Lp</i> ⁺ <i>T</i> ⁻ <i>L</i> ⁻ <i>Th</i> ⁻ <i>Lac</i> ₁ ⁻ <i>Mal</i> ₁ ⁻ <i>Xyl</i> ⁻ <i>Mtl</i> ⁻ <i>Ara</i> ⁻ <i>V</i> ₁ ^r
<i>Gal</i> ₆ ⁻	W-2070	UV, EMB galactose	<i>F</i> ⁺ <i>Lp</i> ⁺ <i>P</i> ⁻ <i>G</i> ⁻
<i>Gal</i> ₇ ⁻	W-583	UV, EMB galactose	<i>F</i> ⁻ <i>Lp</i> ⁺ <i>T</i> ⁻ <i>L</i> ⁻ <i>Th</i> ⁻ <i>Lac</i> ₁ ⁻ <i>Mal</i> ₁ ⁻ <i>Xyl</i> ⁻ <i>Mtl</i> ⁻ <i>Ara</i> ⁻ <i>V</i> ₁ ^r
<i>Gal</i> ₈ ⁻	W-1210	UV, EMB lactose	<i>F</i> ⁺ <i>Lp</i> ⁺ <i>M</i> ⁻ <i>Lac</i> ₁ ⁻ <i>V</i> ₁ ^r

¹ *F* compatibility factor; *M*, *T*, *L*, *Th*, *P*, *G*: auxotrophic markers for methionine, threonine, leucine, thiamin, proline, glycine. *Lac*, *Mal*, *Xyl*, *Mtl*, *Ara*; fermentation of lactose, maltose, D-xylose, mannitol, and L-arabinose. *V*₁^r: resistance to phages *T*₁ and *T*₂. (LEDERBERG *et al.* 1951; LEDERBERG, CAVALLI, and LEDERBERG 1952).

² Each *Gal* locus in this series is referable to a unique mutational event, no recurrences having yet been identified.

³ *Mal*₁⁻, maltose nonfermentation, is associated with nonadsorption of lambda as a pleiotropic effect (E. LEDERBERG 1955). This effect was formerly described as *Lp*₂⁺.

but closely linked loci. These markers were also transferred to other experimental stocks by transduction. The symbols have been explained elsewhere (LEDERBERG and LEDERBERG 1953). As before *Lp*⁺, *Lp*^r and *Lp*^s refer to three allelic states, respectively; lysogenic for the phage lambda, immune and sensitive. Where required, *Lp*^s derivatives were obtained by irradiating *Lp*⁺ stocks with UV. *Lp*^s stocks were converted to *Lp*⁺ (lysogenized) by exposing them to lambda. The infected clones must be carefully purified by serial colony isolation to insure a separation of stably lysogenic from infected sensitive subclones (LEDERBERG and LEDERBERG 1953).

METHODS

The basic technique is the selection and classification of *Gal* types on an indicator medium such as EMB galactose agar. Transduction was accomplished by mixing cells of one genotype with phage from another on agar plates as described previously (MORSE, *et al.* 1956). Two kinds of lysates were used: HFT (high frequency of transduction) from syngenetic bacteria, and LFT (low frequency of transduction) from nonsyngenetic bacteria. The HFT lysates were so active that *Gal*⁺ transformation was readily detected by cross-brushing them with loopfuls of *Gal*⁻ bacteria (fig. 1). Conversely, *Gal*⁻ types could be obtained by growing *Gal*⁺ together with the appropriate *Gal*⁻ HFT lysate, and streaking out the mixtures on EMB galactose agar.

Washed suspensions of compatible, auxotrophic bacteria were crossed on minimal agar (LEDERBERG, CAVALLI, and LEDERBERG 1953). Rare galactose positive recombinants were detected on a minimal indicator medium, EMS galactose agar (J. LEDERBERG 1950).

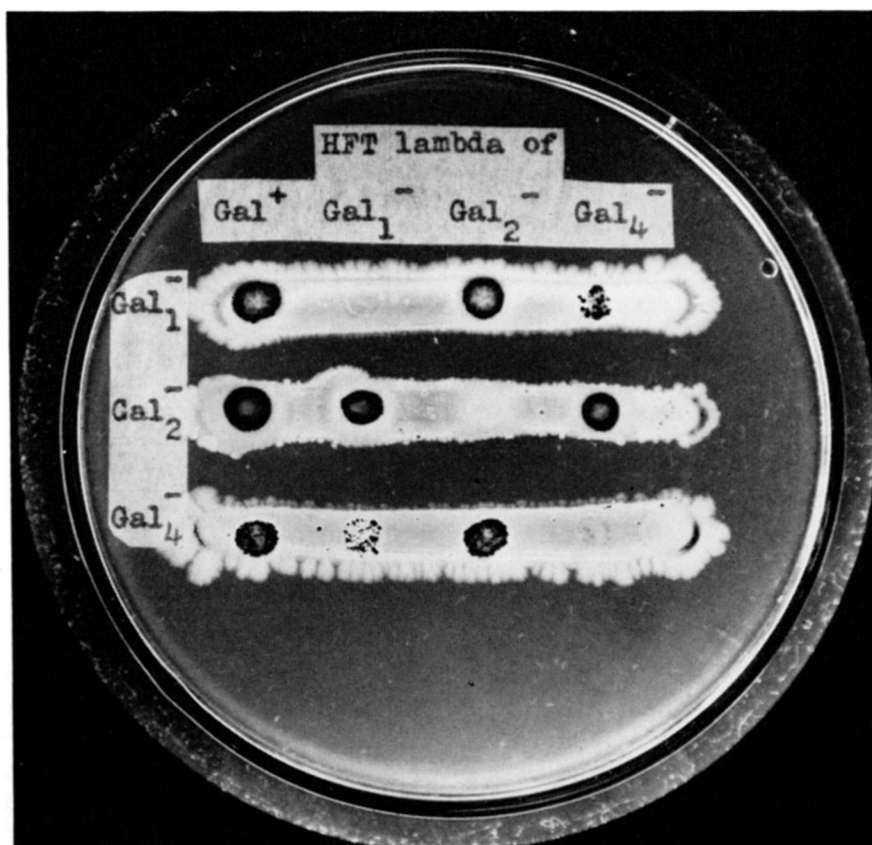


FIGURE 1.—Homology or “allelism” tests. Loopfuls of HFT lambda were spotted on cross streaks of galactose negative cultures and the plate incubated at 37 C for 48 hours. The genotypes of the cultures are given at left, the source of HFT lambda at the top. Each negative culture was transformed to a galactose positive phenotype by HFT lambda from *Gal*⁺ and non-allelic *Gal*[−] cultures, but was not transformed by HFT lambda from its own *Gal* type. Note the lower yields of papillae in the *Gal*₁[−], *Gal*₄[−] interactions. The plate shown contains a modified MacConkey’s medium containing galactose instead of lactose. Routinely EMB galactose agar is used for homology tests, and with results similar to those shown, but because of its better photographic qualities (better contrast) MacConkey’s was chosen for illustration.

EXPERIMENTAL RESULTS

Characteristics of Gal[−] mutants

The *Gal*[−] mutants of table 1 have the common trait of a negative (translucent, near white) reaction on EMB galactose agar. The *Gal*⁺ type reacts by the deposition of an opaque, near purple, stain in the colony (see fig. 3, MORSE *et al.* 1956). Similarly, in fermentation tubes with galactose and bromcresol purple in peptone broth, the *Gal*[−] types remain negative (alkaline, no gas) for several days, while *Gal*⁺ inocula give strong positive reactions (acid, gas) within 24 hours. Both types ferment glucose promptly. A detailed biochemical analysis of the mutants is currently under

way in KALCKAR's laboratory (KURAHASHI, unpublished; KALCKAR *et al.* 1956). Four enzymes, (1) galactokinase, (2) UDP (uridine diphospho)-transferase, (3) UDP-Gal-4-epimerase, and (4) UDP-Glu-pyrophosphatase are known to be involved in the utilization of galactose. *Gal*₁⁻ and other mutants have been found to be deficient in enzyme (2) and *Gal*₂⁻ deficient in enzyme (1).

As shown in table 1, many of the *Gal*⁻ mutants were first recognized by their modification of lactose fermentation, and detected on EMB lactose agar as stable variants of *Lac*⁻ *mutabile* stocks (E. LEDERBERG 1952). The mutants are indistinguishable on EMB galactose agar but differ slightly in their effects on lactose fermentation; *Gal*₁⁻, *Gal*₄⁻ and *Gal*₇⁻ show a weaker *Lac*⁺ phenotype on EMB lactose agar than do *Gal*₂⁻ and *Gal*₈⁻.

The *Gal*⁻ stocks also differ in their spontaneous revertibility to the galactose-positive phenotype, as shown by counts of papillae on confluent plates of EMB galactose agar. As recorded for control plates in transduction assays (tables 1, 2, 7, MORSE *et al.* 1956) *Gal*₄⁻ will usually give about 40 spontaneous papillae per plate, while *Gal*₁⁻ gives about 1 per plate of about 10¹⁰ cells, and the other mutants are intermediate. The rates of mutation have not been precisely measured, and the quoted figures do not distinguish mutant clones in the inocula from plate mutations. Whether the qualitative differences in revertibility are determined by the mutant loci or by modifiers has not been explicitly tested. Furthermore, many of the apparent reversions may be changes at loci other than the *Gal*⁻ involved. Throughout these experiments, control platings have been stressed to minimize confusion from spontaneous reversals of phenotype. When these must be rigorously excluded, double mutants, e.g., *Gal*₁⁻ *Gal*₄⁻ may be used as they have not yet been found to give galactose-positive reversions.

In addition to the distinct galactose-negative mutants just summarized, a variety of weak positive phenotypes have been noted. These are typified by "*Gal*₅⁻", which was unfortunately incorporated in an important series of multiple marker stocks of *E. coli* K-12, W-677 and W-1177 (LEDERBERG *et al.* 1951). These lines originally carried *Gal*₇⁻, but when this was found to interfere with the expression of the *Lac* marker, a phenotypic reversal was selected. After UV treatment, the *Gal*₅⁻ mutant was then obtained, and was chosen for further pedigrees because it did not interfere with *Lac*, although it later proved to be a slow positive. These stocks have evidently accumulated several modifiers, and crosses involving them have given such a variety of galactose phenotypes (cf. contrasting designations by WOLLMAN 1953; CLOWES and ROWLEY 1954) that their further use, particularly where the *Gal*—*Lp* region is involved, should be discouraged. *Gal*₅⁻ itself is not closely linked to the other *Gal* mutants and is not subject to transduction by lambda.

Differentiation of the Gal loci by crosses and by transduction

The most direct means of testing for the allelism of two *Gal*⁻ mutants is to search for galactose positive recombinations. As a necessary control on this procedure, *Gal*⁻ "self crosses" (e.g., *Gal*₁⁻ by *Gal*₁⁻) were also conducted wherever possible: in these crosses no *Gal*⁺ recombinants were detected (table 2). More extensive tests may well be expected to yield some galactose positives by spontaneous reversion,

TABLE 2
Self-crosses of *Gal*⁻ mutants

Cross ¹	Number of recombinants	
	<i>Gal</i> ⁺ prototrophs ^a	Total prototrophs ^b
<i>Hfr M</i> ⁻ × <i>F</i> ⁻ <i>T</i> ⁻ <i>L</i> ⁻ <i>Th</i> ⁻		
<i>Gal</i> ₁ ⁻ × <i>Gal</i> ₁ ⁻	0	4,200,000
<i>Gal</i> ₂ ⁻ × <i>Gal</i> ₂ ⁻	0	140,000
<i>Gal</i> ₄ ⁻ × <i>Gal</i> ₄ ⁻	0	800,000
<i>Gal</i> ₆ ⁻ × <i>Gal</i> ₆ ⁻	0	160,000
<i>Gal</i> ₇ ⁻ × <i>Gal</i> ₇ ⁻	0	120,000

¹ Approximately 10⁸ cells of each parent were grown together in broth for three hours, after which time aliquots were a) plated on a minimal medium whose sole carbon source was galactose; b) diluted and plated on a minimal medium with glucose as the carbon source.

which is probably the chief limitation to the resolving power of this method. Pair-wise crosses of different *Gal*⁻ gave a small fraction (about one per thousand) of *Gal*⁺ prototrophs, which indicates that they are different but closely linked loci (table 3).

The loci *Gal*₁, *Gal*₂, and *Gal*₄ have been most widely used, and have been test-crossed in all combinations; a few additional crosses are also listed in table 3. The loci have also been differentiated by transduction; this is shown diagrammatically in table 4. With HFT lysates, several million homology tests may be performed on a single EMB galactose agar plate (fig. 1). Therefore, HFT lysates, rather than LFT, have been used as the standard reagents of the allelism tests.

The transduction data summarized in table 4 therefore permit seven loci to be distinguished, despite occasional gaps. These distinctions are entirely consistent with those of testcrosses, insofar as these have been made. About 50 additional *Gal*⁻ mutants which can be transformed by various lysates have been isolated, but have not been completely analysed.

Segregation from syngenotes

Many of the *Gal*⁺ clones formed by the transduction, *Gal*⁺ —× *Gal*⁻, i.e., *Gal*⁺ lysate and *Gal*⁻ cells, are unstable for this trait (see also MORSE *et al.* 1956, fig. 3 and table 4). The instability is attributed to segregation because the *Gal*⁻ clones which reappear are always the same type as the original *Gal*⁻ recipient (table 5). To generalize the test as far as possible, each *Gal*⁻ segregant was picked from a heterogenetic clone which had been isolated from an independent transductional event. The segregants were typed by their reactions with various HFT reagents. Double mutants are characterized by their failure to react with either of two reagents, though they respond to *Gal*⁺ and other *Gal*⁻ lysates. Throughout this paper, the stated formula has been inferred from this test, together with any other measures that may be indicated.

In addition, the *Gal*⁺ clones obtained by *Gal*₁⁻ —× *Gal*₂⁻, etc., are often unstable and can give more information on the segregation process. As before, each *Gal*⁻ segregant was referable to an individual transduction clone to give a composite picture of heterogenote behavior. The tests of these segregants are given in table 5.

TABLE 3
Inter-crosses of *Gal*⁻ mutants

A.	Cross ¹	Number of recombinants	
		<i>Gal</i> ⁺ prototrophs ^a	Total prototrophs ^b
	<i>Hfr M</i> ⁻ × <i>F</i> ⁻ <i>T</i> ⁻ <i>L</i> ⁻ <i>Th</i> ⁻		
	<i>Gal</i> ₁ ⁻ × <i>Gal</i> ₂ ⁻	11	15,000
	<i>Gal</i> ₁ ⁻ × <i>Gal</i> ₃ ⁻	3	82,000
	<i>Gal</i> ₁ ⁻ × <i>Gal</i> ₄ ⁻	3	90,000
	<i>Gal</i> ₁ ⁻ × <i>Gal</i> ₆ ⁻	7	102,000
	<i>Gal</i> ₁ ⁻ × <i>Gal</i> ₇ ⁻	5	14,000
	<i>Gal</i> ₂ ⁻ × <i>Gal</i> ₁ ⁻	152	190,000
	<i>Gal</i> ₂ ⁻ × <i>Gal</i> ₄ ⁻	66	400,000
	<i>Gal</i> ₂ ⁻ × <i>Gal</i> ₆ ⁻	16	93,000
	<i>Gal</i> ₃ ⁻ × <i>Gal</i> ₆ ⁻	36	180,000
	<i>Gal</i> ₄ ⁻ × <i>Gal</i> ₁ ⁻	36	108,000
	<i>Gal</i> ₄ ⁻ × <i>Gal</i> ₂ ⁻	231	125,000
	<i>Gal</i> ₄ ⁻ × <i>Gal</i> ₆ ⁻	28	174,000
	<i>Gal</i> ₄ ⁻ × <i>Gal</i> ₇ ⁻	10	160,000
	<i>Gal</i> ₄ ⁻ × <i>Gal</i> ₈ ⁻	83	210,000
	<i>Gal</i> ₆ ⁻ × <i>Gal</i> ₁ ⁻	7	180,000
	<i>Gal</i> ₆ ⁻ × <i>Gal</i> ₂ ⁻	36	33,000
	<i>Gal</i> ₆ ⁻ × <i>Gal</i> ₃ ⁻	248	72,000
	<i>Gal</i> ₆ ⁻ × <i>Gal</i> ₇ ⁻	13	80,000
	<i>Gal</i> ₇ ⁻ × <i>Gal</i> ₁ ⁻	5	110,000
	<i>Gal</i> ₇ ⁻ × <i>Gal</i> ₂ ⁻	12	19,000
	<i>Gal</i> ₇ ⁻ × <i>Gal</i> ₃ ⁻	127	66,000
	<i>Gal</i> ₇ ⁻ × <i>Gal</i> ₄ ⁻	1	102,000
	<i>Gal</i> ₈ ⁻ × <i>Gal</i> ₆ ⁻	130	81,000
B.	Cross ²	Number of recombinants	
		<i>Gal</i> ⁺ prototrophs	Total prototrophs
	<i>Gal</i> ₁ ⁻ × <i>Gal</i> ₂ ⁻	11	13,700
	<i>Gal</i> ₁ ⁻ × <i>Gal</i> ₃ ⁻	2	1,600
	<i>Gal</i> ₁ ⁻ × <i>Gal</i> ₄ ⁻	38	11,600
	<i>Gal</i> ₁ ⁻ × <i>Gal</i> ₇ ⁻	2	7,600
	<i>Gal</i> ₂ ⁻ × <i>Gal</i> ₄ ⁻	13	7,400
	<i>Gal</i> ₂ ⁻ × <i>Gal</i> ₅ ⁻	3	18,400
	<i>Gal</i> ₃ ⁻ × <i>Gal</i> ₈ ⁻	14	17,064
	<i>Gal</i> ₄ ⁻ × <i>Gal</i> ₈ ⁻	44	10,000

¹ See footnote, table 2, for method.

^a See footnote ¹, table 2.

^b See footnote ¹, table 2.

² These data are pooled from a number of experiments with different mating type combinations. The crosses were conducted on EMS galactose agar, where galactose-positive and negative prototroph recombinants are scored on the same plates. These methods require further standardization before the apparent recombination fractions can be given precise *quantitative* meaning.

TABLE 4
Differentiation of the *Gal*⁻ mutants by transduction

Recipient cells	Lysates ¹							
	<i>Gal</i> ⁺	<i>Gal</i> ₁ ⁻	<i>Gal</i> ₂ ⁻	<i>Gal</i> ₃ ⁻	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₅ ⁻	<i>Gal</i> ₇ ⁻	<i>Gal</i> ₈ ⁻
<i>Gal</i> ₁ ⁻	+	-	+	+	+	+	+	+
<i>Gal</i> ₂ ⁻	+	+	-	+	+	+	+	+
<i>Gal</i> ₃ ⁻	+	+	+	-	+	+	+	+
<i>Gal</i> ₄ ⁻	+	+	+	+	-	+	+	+
<i>Gal</i> ₅ ⁻	+	+	+	+	+	-	+	+
<i>Gal</i> ₇ ⁻	+	+	+	+	+	+	-	+
<i>Gal</i> ₈ ⁻	+	+	+	+	+	+	+	-

¹ A suspension of each type of recipient cell was cross-brushed against the indicated HFT lysates on EMB galactose agar. + indicates that galactose-positive transformations were observed, - that they were not. For *Gal*₃⁻ and *Gal*₈⁻, only LFT lysates were available and the reactions were recorded from platings of 0.1 ml lysate plus 0.1 ml cell suspension.

Three kinds of exogenote are considered: (1) carrying *Gal*⁺, (2) carrying non-allelic *Gal*⁻, (3) carrying two *Gal*⁻ loci, non-allelic with the endogenotic *Gal*⁻. The segregation patterns were:

Exogenote carrying	<i>Gal</i> ⁻ segregant type (percent)		
	Endogenotic	Exogenotic	Recombinant types
<i>Gal</i> ⁺	100	0	0
Non-allelic <i>Gal</i> ⁻	88	11	1
Double <i>Gal</i> ⁻	88	10	2

In every combination shown in table 5, the *Gal*⁻ types which are recovered correspond exactly to the types which entered into the transduction, or to recombinations of them. The order of frequency of the segregant types was endogenotic > exogenotic > recombinant. The *Gal*⁺ clone thus behaves as if the recipient cell had received a small fragment of chromosome containing the *Gal*⁺ genes (and only these since unrelated markers are not changed). Segregation from the resulting partial diploid (heterogenote) is biased, and more frequently restores the endogenotic *Gal*⁻ phenotype. Less frequently the exogenotic *Gal*⁻ is recovered, either alone or recombined as a double *Gal*⁻. To complete the segregation pattern, stable *Gal*⁺ segregants should be demonstrated. These are distinguished less readily than *Gal*⁻ and so have not been systematically enumerated but have been isolated incidentally to a number of experiments.

Genotypic formulae for syngenotes can be written *Endogenote*/_{ex}*Exogenote*, e.g., *Gal*₂⁻ *Gal*₁⁺ *Gal*₆⁺/_{ex} *Gal*₂⁺ *Gal*₁⁻ *Gal*₆⁻ for the last item of table 5. By abbreviating the same symbols, the three kinds of syngenotes summarized above can be styled as ++-/_{ex}+++ , +-+/_{ex}+-+ , and +-+/_{ex}--+ . Until relevant data are available, no implications concerning gene sequence should be read into the formulae.

The data of table 5 show no obvious difference between reciprocal transductions;

TABLE 5
Segregation from the syngenotes

Syngenote; Recipient $Gal_{\bar{y}}$ Donor $Gal_{\bar{x}}$ or Gal^+		Number of segregants observed with:			
		$Gal_{\bar{y}}$	$Gal_{\bar{x}}$ or Gal^+	$Gal_{\bar{x}} Gal_{\bar{y}}$	Total
<i>endogene/ exogene</i>		<i>endogenetic</i>	<i>exogenetic</i>	<i>crossover</i>	
$Gal_1^- Lp^s$	Gal^+	9	0	0	9
Lp^+	Gal^+	33	0	0	33
$Gal_2^- Lp^s$	Gal^+	16	0	0	16
Lp^+	Gal^+	20	0	0	20
$Gal_4^- Lp^s$	Gal^+	31	0	0	31
Lp^+	Gal^+	20	0	0	20
Lp^r	Gal^+	29	0	0	29
$Gal_8^- Lp^s$	Gal^+	29	0	0	29
Lp^+	Gal^+	15	0	0	15
		201 (100%)			201
$Gal_1^- Lp^s$	Gal_2^-	1	0	0	1
	Gal_8^-	6	1	0	7
Lp^+	Gal_2^-	36	6	0	42
	Gal_8^-	18	3	0	21
$Gal_2^- Lp^+$	Gal_1^-	14	3	2	19
	Gal_4^-	9	7	0	16
$Gal_4^- Lp^s$	Gal_2^-	18	3	0	21
	Gal_8^-	17	2	0	19
Lp^+	Gal_2^-	16	3	0	19
Lp^r	Gal_2^-	15	3	0	18
$Gal_8^- Lp^s$	Gal_1^-	40	1	0	41
	Gal_4^-	42	1	1	44
Lp^+	Gal_1^-	19	2	0	21
	Gal_4^-	22	1	0	23
		273 (87.5%)	36 (11.5%)	3 (0.9%)	312
$Gal_8^- Lp^{+1}$	$Gal_1^- Gal_4^-$	135	14	3	152
	$Gal_1^- Gal_7^-$	29	1	0	30
$Gal_2^- Lp^+$	$Gal_6^- Gal_7^-$	18	2	1	21
	$Gal_1^- Gal_6^-$	12	4	0	16
		194 (88.5%)	21 (9.6%)	4 (1.8%)	219

¹ In these transductions, involving three factors, $Gal_{\bar{x}}$ refers to both mutant loci taken together; the crossovers are other combinations.

nor is there any effect of the Lp allele of the recipient on the segregation pattern of the resulting syngenotes. As noted previously (MORSE *et al.* 1956) all transduction clones derived from Lp^+ recipients were lysogenic; from Lp^s recipients, Lp^+ or Lp^r ; and from Lp^r recipients, usually Lp^r . The segregational behavior of Lp alleles is still under study and will be taken up elsewhere.

The tests for Gal type were based on the pattern of transduction by lysates from known Gal^- cultures. As additional checks on the transductional test for Gal type

TABLE 6
Examination of segregants from heterogenotic clones

Heterogenote		Classification ¹ of segregant by:					
		Transduction <i>Gal</i> type	Lysate activity <i>Gal</i> type	Testcross to:			
				Endogenotic <i>Gal</i> ⁻		Exogenotic <i>Gal</i> ⁻	
				<i>Gal</i> ⁺	total	<i>Gal</i> ⁺	total
<i>Gal</i> ₂ ⁻ / <i>ex Gal</i> ₂ ⁺	1.	<i>Gal</i> ₂ ⁻	<i>Gal</i> ₂ ⁻	0	4070		
	2.	<i>Gal</i> ₂ ⁻	<i>Gal</i> ₂ ⁻	0	5384		
	3.	<i>Gal</i> ₂ ⁻	<i>Gal</i> ₂ ⁻	0	2072		
	4.	<i>Gal</i> ₂ ⁻	<i>Gal</i> ₂ ⁻	0	6988		
<i>Gal</i> ₄ ⁻ / <i>ex Gal</i> ₄ ⁺	1.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	896		
	2.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	918		
	3.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	1134		
	4.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	863		
<i>Gal</i> ₄ ⁻ <i>Gal</i> ₂ ⁺ / <i>ex Gal</i> ₄ ⁺ <i>Gal</i> ₂ ⁻	1.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	2786	3	3183
	2.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	2675	2	3471
	3.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	3485	23	5342
	4.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	5952	1	1665
	5.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	5000	1	891
	6.	<i>Gal</i> ₂ ⁻	<i>Gal</i> ₂ ⁻	7	3102	0	1988
	7.	<i>Gal</i> ₂ ⁻	<i>Gal</i> ₂ ⁻	10	4364	0	1187
<i>Gal</i> ₄ ⁻ <i>Gal</i> ₂ ⁺ / <i>ex Gal</i> ₄ ⁺ <i>Gal</i> ₂ ⁻	1.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	16104	0	1389
	2.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	5730	1	164
	3.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	3358	0	202
	4.	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₄ ⁻	0	12848	1	171
	5.	<i>Gal</i> ₂ ⁻	<i>Gal</i> ₂ ⁻	1	11200	0	827
	6.	<i>Gal</i> ₂ ⁻	<i>Gal</i> ₂ ⁻	6	10608	0	718
	7.	<i>Gal</i> ₂ ⁻	<i>Gal</i> ₂ ⁻	3	5000	0	409

¹ Classifications: (1) by transduction, exposure to lysates of known *Gal*⁻; (2) lysate activity, lysate of the segregant on known *Gal*⁻; (3) testcrossing with known *Gal*⁻ cultures, the figures given are *Gal*⁺ prototrophic recombinants and total prototrophic recombinants, scored as per table 3B.

a number of segregants were tested further by two methods: (1) by making lysates of them and plating the lysate with known *Gal*⁻ cultures, (2) test crossing against known *Gal*⁻ cultures. These further checks on the classification gave perfect agreement with each other and with the transductional test. Cultures of 79 segregants were checked by transduction to known *Gal*⁻, and 26 segregants were testcrossed. The scope of the analysis is indicated by the data in table 6, in which data on segregants tested by both methods are recorded.

The sequence of events in segregation from a single heterogenote could, in principle, be studied in cell pedigrees, but the rate of segregation, about 10^{-3} per division, would make this too laborious an enterprise at present. A number of individual heterogenotes have, however, been studied intensively by plating methods. For example, W2869, *Gal*₄⁻*Gal*₂⁺ / *ex Gal*₄⁺*Gal*₂⁻ was replated after purification, and no more than one *Gal*⁻ segregant tested per segregating *Gal*⁺ colony. This insures the uniqueness of each

segregant. From a total of 112 colonies, the segregant types were 85 Gal^- ; 26 Gal_2^- and 1 $Gal_2^- Gal_4^-$, which are endogenotic, exogenotic and recombinant, respectively.

Syngenotes are distinguished from haploids (nonsyngenotes) primarily by their genetic complexity, which is revealed by segregation. A second distinction, with technical import for further analysis, is their behavior as transductional donors. As noted previously (MORSE *et al.* 1956) the lysates from heterogenotic cultures show a very high frequency of transduction. Every Lp^+ heterogenote which has been tested has given a lysate showing HFT behavior, i.e., not less than one transduction per hundred plaque-forming particles, and often approaching one per one. Clones which give HFT lysates will be referred to as HFT⁺. As will be noted later, HFT⁺ behavior has served in turn as an auxiliary criterion for homogenotes, in which segregation is less readily observed.

HFT lysates from a number of double heterogenotes, e.g., $Gal_1^- Gal_2^+ / exGal_1^+ Gal_2^-$, have been assayed on various Gal^- types, with the results depicted in table 7. The exogenote is preferentially included in the phage which matures in the syngenotes. In view of the incidence of non-syngenotic segregants, which are known to generate much larger bursts of LFT phage than syngenotes, the true efficiency of phage from heterogenotes is systematically underestimated. The estimation of preferential inclusion of exogenotes is also complicated by the incidence of syngenotic crossovers, as described in the following section.

Homogenotic segregants

Three *a priori* possibilities for the constitution of Gal^- segregants may be considered (the first two of which have been realized): (1) reduced haploids; (2) unreduced syngenotes, which have become homogenotic for one or more Gal^- loci; (3) diploids homozygous for one or more Gal^- loci (these might arise by secondary non-disjunction). If segregants occur whose genotypes consist only of exogenotes, we assume they would be inviable. Each of the events might or might not be preceded by crossing over.

Two methods are available for distinguishing homogenotic ($Gal^- / exGal^-$) from haploid, nonsyngenotic ($Gal^- /$) segregants. Reverse mutation from Gal^- to Gal^+ would convert a homogenote to a segregating heterogenote, $Gal^- / exGal^-$ to $Gal^+ / exGal^-$ or $Gal^- / exGal^+$. A haploid Gal^- would revert to a haploid Gal^+ , which would not segregate for galactose fermentation; this supposition is supported without exception by tests of at least 100 reversions from type Gal^- cultures, usually obtained as papillae from control platings in transduction assays (see, e.g., tables 1, 2, 7, MORSE *et al.* 1956).

In addition, by analogy with heterogenotes, homogenotic cultures should be HFT⁺. Haploid segregants like all of the type haploid stocks which have been tested should be LFT⁺. Altogether, 77 segregants from 18 different heterogenotes were screened initially by the reversion test. For each test from one to ten independent reversions was selected from each Gal^- segregant clone. Two of the 77 (2.6%) gave segregating reversion clones and are tentatively considered to be homogenotic. Both segregants happen to come from W2869 ($Gal_1^- Gal_2^+ / exGal_1^+ Gal_2^-$) and are themselves $Gal_2^- / exGal_2^-$ homogenotes. From one of these clones, both of two reversions were unstable; from

TABLE 7
Lysates of heterogenotes

Heterogenote		Assay of lysate of heterogenote for:			Ratio exo:endo
Endogenote	Exogenote	Phage ¹	Exogenote <i>Gal</i> ¹⁺²	Endogenote <i>Gal</i> ¹⁺³	
<i>Gal</i> ₁ ⁻	<i>Gal</i> ₂ ⁻	7200	120	1	120
<i>Gal</i> ₁ ⁻	<i>Gal</i> ₂ ⁻	1	1.8	0.06	30
<i>Gal</i> ₂ ₁	<i>Gal</i> ₁ ⁻	620	150	43	3.5
<i>Gal</i> ₂ ⁻	<i>Gal</i> ₄ ⁻	730	25	0.28	89
<i>Gal</i> ₂ ⁻	<i>Gal</i> ₄ ⁻	21	14	1.2	11

¹ Plaques on *Lp*⁺ culture.
² Papillae on *Lp*⁺ endogenotic *Gal*⁻ culture.
³ Papillae on *Lp*⁺ exogenotic *Gal*⁻ culture.

the other, one reversion was unstable, the other stable. The last observation may be attributed to a suppressor mutation in another region, further segregation in the homogenotic clone, or to the lesser frequency of *Gal*⁻ (exogenotic) segregants from the potential *Gal*⁺/*exGal*⁻ type of reversion. Consequently, the total estimation of the incidence of homogenotic segregants is probably low, especially for segregants of which only one or a few reversions have been examined.

Both of the inferred *Gal*⁻ homogenotic segregants were tested and found to be HFT⁺, a result which bolsters the confidence that may be placed in this more convenient test. To simplify the test, a suspension of *Gal*⁻ cells was held in a loop under a UV lamp, then spotted on a plate previously spread with indicator *Gal*⁻ cells. Alternatively, such a plate may be irradiated after it has been spotted. Low doses of UV are used, which leave enough bacterial survivors, but release sufficient phage to distinguish HFT⁺ from LFT⁺. To classify its *Gal* type, the segregant is tested on indicators which correspond to the components of its parental heterogenote. For example, lysates of *Gal*⁻ segregants from W-2869 and the heterogenotes listed in table 7 were tested on *Gal*₂⁻ and *Gal*₄⁻ indicators.

For a study of the incidence of homogenotic segregants, six independent heterogenotes were isolated from *Gal*₄⁻ —× *Gal*₂⁻. Individual segregants of each heterogenote were tested for HFT⁺ as shown in table 8. Altogether, of 104 segregants tested, 20 were homogenotic. However, the heterogenotes were quite disparate: one of them accounts for 16 homogenotes, and these were all of the exogenotic type.

TABLE 8
Homogenotic segregants (detected as HFT⁺)

Number of homogenotes	<i>Gal</i> ₂ ⁺ <i>Gal</i> ₄ ⁻ / <i>ex Gal</i> ₂ ⁺ <i>Gal</i> ₄ ⁻ heterogenotes					
	293-1	293-2	293-3	293-11	293-12	293-13
<i>Gal</i> ₂ ⁻ / <i>ex Gal</i> ₂ ⁻	1	2	0	1	0	0
<i>Gal</i> ₄ ⁻ / <i>ex Gal</i> ₄ ⁻	0	0	0	0	16	0
Total tested.....	11	11	11	11	49	11

TABLE 9
Observations on homogenotic clones

Homogenote			LFT ⁺ segregant	
Phenotype	Derived from	Galactose-positive reversions segregating/total	Phenotype	Galactose-positive reversions segregating/total
Gal ₁ ⁻	1 ⁻ 2 ⁺ / <i>ex</i> 1 ⁺ 2 ⁻	4/5	Gal ₁ ⁻	0/8
Gal ₂ ⁻	2 ⁻ 4 ⁺ / <i>ex</i> 2 ⁺ 4 ⁻	(1) 4/4	Gal ₂ ⁻	—*
		(2) 2/3	Gal ₂ ⁻	—
		(3) 2/3	Gal ₂ ⁻	—
		(4) 3/4	Gal ₂ ⁻	—
		(5) 4/6	Gal ₂ ⁻	0/5
Gal ₂ ⁻	1 ⁻ 2 ⁺ / <i>ex</i> 1 ⁺ 2 ⁻	(1) 12/12	Gal ₁ ⁻ Gal ₂ ⁻	none obtained
		(2) 12/12	Gal ₂ ⁻	0/12
		(3) 12/12	Gal ₁ ⁻ Gal ₂ ⁻	none obtained
Gal ₂ ⁻	2 ⁺ 4 ⁻ / <i>ex</i> 2 ⁻ 4 ⁺	(1) 10/18	Gal ₂ ⁻	—
		(2) 2/2	—	—
		(3) 1/2	Gal ₂ ⁻	—
Gal ₄ ⁻	2 ⁻ 4 ⁺ / <i>ex</i> 2 ⁺ 4 ⁻	(1) 1/6	—	—
		(2) 1/6	—	—
		(3) 2/4	—	—
		(4) 1/1	—	—
		(5) 3/4	—	—
Gal ₆ ⁻	2 ⁻ 6 ⁺ / <i>ex</i> 2 ⁺ 6 ⁻	2/2	Gal ₆ ⁻	0/3
Gal ₇ ⁻	2 ⁺ 7 ⁻ / <i>ex</i> 2 ⁻ 7 ⁺	8/14	Gal ₇ ⁻	0/7

* — signifies not examined.

Further segregation from the HFT⁺ homogenotes cannot be detected by instability of the *Gal* phenotype. It is, however, reflected (1) in the segregation of *Gal*⁺/*Gal*⁻ heterogenotes which result from reversion of one *Gal*⁻ allele, (2) in the spontaneous occurrence of LFT⁺ derivatives, whose *Gal*⁺ reversions do not segregate (table 9).

The HFT lysates from homogenotes may be exploited as pure reagents for *Gal* typing. The homogenotes for this purpose have been obtained from two-locus heterogenotes, in which the desired *Gal*⁻ factor was either endo- or exogenotic (table 9). At the present time, HFT lysates have been obtained for *Gal*₁, *Gal*₂, *Gal*₄, *Gal*₆, and *Gal*₇ but not for *Gal*₃ or *Gal*₈. In the search for the appropriate homogenotes, 385 segregants were tested, and 24 homogenotes were found. However, for the immediate purpose, the segregants had been tested on only one indicator, and some homogenotes might have been missed.

The homogenotes so far considered are not necessarily related to crossing over between the *Gal* markers. For example, the first line of table 8 shows *Gal*₁⁻*Gal*₂⁺/*exGal*₁⁺*Gal*₂⁻ giving *Gal*₁⁻*Gal*₂⁺/*exGal*₁⁻*Gal*₂⁺, while the seventh line shows the alternative *Gal*₁⁺*Gal*₂⁻/*exGal*₁⁺*Gal*₂⁻ homogenote. This formulation is based on the *Gal* typing of the LFT⁺ segregants. However, segregants of the form *Gal*₁⁻*Gal*₂⁻/*exGal*₁⁺*Gal*₂⁻ have also been identified. They give the same typing reactions (i.e., *Gal*₂⁻) to HFT reagents as *Gal*₁⁺*Gal*₂⁻/*exGal*₁⁺*Gal*₂⁻, but they continue to segregate to give *Gal*₁⁻*Gal*₂⁻ types. From these results we would infer that segregation may coincide with crossing

over, whether syngenotic or non-syngenotic types result. Obviously, the crossover syngenotes could result only from a system of crossing over involving more than two strands.

On a number of structural hypotheses, crossing over and segregation in a syngenote might lead to diploidization and homozygosity for markers not initially involved in transduction. This can be tested by the segregation of reverse mutations of the indicated marker as has already been described for *Gal* homogenotes, and previously used for the discrimination of hemi- from homozygous diploids (LEDERBERG *et al.* 1951). The markers: *Lac*₁, *Mal*₁, *Xyl*₂, *Ara*₂, have been used for this purpose. The tests, while not very extensive, indicate that homozygosity for these markers is not correlated with segregation for *Gal* from heterogenotes.

Segregation from heterogenotes showing position effect

Under the usual assay conditions, some transductional combinations of one *Gal*⁻ with another give galactose positive clones with the same frequency and rapidity as transductions from *Gal*⁺ to *Gal*⁻. *Gal*₁⁻ —× *Gal*₂⁻, *Gal*₂⁻ —× *Gal*₄⁻, and their reciprocals are included in this category; *Gal*⁺ —× *Gal*₁⁻*Gal*₄⁻ is equally prompt. On the other hand, *Gal*₁⁻ —× *Gal*₄⁻ and *Gal*₄⁻ —× *Gal*₁⁻ give lower yields and a delayed development of positive clones (fig. 1). As we shall see this discrepancy can be explained by the galactose-negative phenotype of the *I*⁺*4*⁻/*exI*⁺*4*⁻ and *I*⁺*4*⁺/*exI*⁺*4*⁻ heterogenotes, which can be isolated from appropriate mixtures of cells and HFT lysate on EMB galactose agar. The heterogenotes can be distinguished from the nonsyngenotic *Gal*⁻ recipients by the frequent development of positive papillae and sectors after two to three days incubation. The heterogenotes *I*⁺*4*⁻/*exI*⁺*4*⁺ and *I*⁺*4*⁺/*exI*⁺*4*⁻ have also been prepared and found to be galactose positive. The various combinations and phenotypes can thus be summarized:

trans	trans	cis	cis
+ -/ <i>ex</i> - + negative	- +/ <i>ex</i> + - negative	- -/ <i>ex</i> + + positive	+ +/ <i>ex</i> - - positive

The pattern is diagnostic of a cis-trans position effect.

The constitutions of these heterogenotes are primarily inferred from their immediate parentage, e.g. *I*⁺*4*⁻/*exI*⁺*4*⁻ is considered to be the typical result of *I*⁺*4*⁻ —× *I*⁺*4*⁻. For the present we shall be concerned principally with their segregational behavior, which is consistent with their inferred constitution and follows the same lines as segregation in other heterogenotes (table 10). The interactions of these and other *Gal* loci in heterogenotes and heterozygotes are being studied in more detail for presentation elsewhere.

The galactose-positive cis heterogenote *I*⁺*4*⁻/*exI*⁺*4*⁺ was obtained from *Gal*⁺ —× *Gal*₁⁻*Gal*₄⁻. (The latter was isolated as a segregant from a heterogenote *I*⁺*4*⁻/*exI*⁺*4*⁺, table 10). The opposite arrangement, *I*⁺*4*⁺/*exI*⁺*4*⁻ could not be directly selected, but was obtained as the galactose positive heterogenote *I*⁺*4*⁺*8*⁻/*exI*⁺*4*⁻*8*⁺, by the transduction *Gal*₁⁻*Gal*₄⁻ —× *Gal*₈⁻. The trans-heterogenotes were isolated as already de-

TABLE 10
Segregation from heterogenetic clones showing position effect

A. The <i>cis</i> -position, galactose positive clones					
Heterogenote		Segregants with <i>Gal</i> genotype			
Endogenote	Exogenote	<i>Gal</i> _s ⁻	<i>Gal</i> ₁ ⁻ <i>Gal</i> ₄ ⁻	<i>Gal</i> ₁ ⁻	Total
<i>I</i> ⁻ <i>4</i> ⁻	<i>I</i> ⁺ <i>4</i> ⁺	0	24	0	24
<i>8</i> ⁻ <i>I</i> ⁺ <i>4</i> ⁺	<i>8</i> ⁺ <i>I</i> ⁻ <i>4</i> ⁻	135	14	3	152

B. The <i>trans</i> -position, galactose negative clones					
Heterogenote		Segregants with <i>Gal</i> genotype			
Endogenote	Exogenote	<i>Gal</i> ₁ ⁻	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₁ ⁻ <i>Gal</i> ₄ ⁻	Total
<i>I</i> ⁻ <i>4</i> ⁺	<i>I</i> ⁺ <i>4</i> ⁻	21	3	0	24
<i>I</i> ⁺ <i>4</i> ⁻	<i>I</i> ⁻ <i>4</i> ⁺	4	14	0	18

C. The galactose positive clones (crossover syngenotes) occurring in <i>trans</i> -position clones ¹						
Crossover syngenotes in <i>trans</i> -position heterogenotes with:		Segregants with <i>Gal</i> genotype				
Endogenote	Exogenote	<i>Gal</i> ⁺²	<i>Gal</i> ₁ ⁻	<i>Gal</i> ₄ ⁻	<i>Gal</i> ₁ ⁻ <i>Gal</i> ₄ ⁻	Total
<i>I</i> ⁻ <i>4</i> ⁺	<i>I</i> ⁺ <i>4</i> ⁻	9	2	3	0	14
<i>I</i> ⁺ <i>4</i> ⁻	<i>I</i> ⁻ <i>4</i> ⁺	12	4	4	3	23

¹ After 24–36 hours the *trans*-position galactose negative colonies papillate and show, after re-streaking, galactose positive clones, which may or may not segregate galactose negatives. Each segregant listed was obtained from a separate rearrangement to give the galactose positive phenotype. In addition to the *Gal*⁻ types listed as obtained from the rearrangements, some papillating galactose negatives were also obtained. The latter are presumed to be rearrangements from *++*/*ex* — — to *++*/*ex* — + and — *++*/*ex* + —.

² Clones listed under this column failed to segregate in two streakings. They may have a *++* endogenote or they may be haploid *++*.

scribed. *Gal*⁻ segregants were detected by their failure to papillate. Galactose-positive segregants were ignored for this tabulation, but are considered below. The segregation patterns for these heterogenotes are given in table 5.

In addition, table 10 presents the segregational patterns of galactose-positive derivatives, isolated as papillae or sectors from *trans*-heterogenetic colonies. Some of the galactose-positives failed to segregate in the routine test, and are assumed to carry *I*⁺*4*⁺ crossovers. The segregants might include non-syngenetic haploids, and syngenotes with a *++* endogenote whose segregation would not be readily detected. Segregating galactose-positive clones included types inferred from single segregants *++* — *ex* + +, — *++*/*ex* + +, and — — *ex* + +, as listed in table 10. The "galactose-negative segregants" also included colonies which later papillated, and thus resembled the original *trans*-heterogenotes. These are readily interpreted as *++* — / — + crossover syngenotes, resulting either from — — *ex* + + or *++* — *ex* — — galactose-positive heterogenotes.

DISCUSSION

During the past 30 years, non-sexual processes of genetic recombination have been discovered in a variety of bacterial species: pneumococcus (GRIFFITH 1928; HOTCHKISS 1955; EPHRUSSI-TAYLOR 1955); Hemophilus (ALEXANDER and LEIDY 1951); Neisseria (ALEXANDER and REDMAN 1953); Salmonella spp. (ZINDER and LEDERBERG 1952; BARON 1955; ISEKI 1954; EDWARDS 1955); *Escherichia coli* (LENNOX 1955; JACOB 1955; MORSE *et al.* 1956). The common feature of the examples that have just been cited is the transmission of a small hereditary fragment by a cell-free preparation. The same examples differ in their details, some being mediated by free desoxyribonucleic acid (DNA) and others entailing phage particles as a vector. To emphasize a unitary genetic interpretation of these examples, and to distinguish them from sexual processes, the term transduction (J. LEDERBERG 1952) was proposed for the transfer of genetic fragments. Some workers have since used "transduction" more narrowly, to distinguish phage-mediated from DNA-mediated "transformation", but we are more concerned with the unified interpretation of these phenomena than with the rigidification of the nomenclature.

The principal features of the *E. coli*-lambda system of transduction may be summarized as follows:

1. The transduced markers. A large number of galactose-nonfermenting (*Gal*⁻) mutants have been obtained in *E. coli* K-12. Although these mutants are phenotypically similar, each of the first seven occurrences to be analysed has been related to a distinct locus, separable from the others by recombination tests. These tests have included sexual crosses, as well as reciprocal transductions, with concordant results in each case. The various *Gal*⁻ loci are closely linked to one another and to the *Lp* locus, the determinant of lambda-lysogenicity.

2. Transduction by lambda. Ordinary cultures of lysogenic (*Lp*⁺) bacteria produce lambda sporadically and to a low titer. When these cells are exposed to ultraviolet light (UV) a large proportion of them are induced to yield free lambda, with concomitant lysis of the bacteria (WEIGLE and DELBRÜCK 1951). Occasional particles of "induced lambda" from *Lp*⁺ cells contain the linked *Gal* genes, in addition to the lambda prophage itself. This transduction of *Gal* markers is revealed by the transformation of *Gal*⁻ cells to galactose-positive phenotypes. The specificity of this transformation rules out general mutagenic effects: lysates of *Gal*⁻ cultures do not transform homologous types, but will transform each of the heterologous mutants. No markers other than *Gal* have been found to be transduced by lambda.

That the lambda particle itself is the active component of the lysate is indicated by the correlation of lysogenizing with transducing activity. Lysogenic, immune, and sensitive cultures (*Lp*⁺, *Lp*^r and *Lp*^s, respectively) are all effective recipients of transduction by lambda.

Lambda can also be produced by its lytic growth on *Lp*^s bacteria. In contrast to the "induced lambda" from *Lp*⁺ bacteria, this "lytic lambda" is not demonstrably effective in transduction.

3. Segregation in transformed clones. The transformed clones resulting from transduction fall into two categories. About a third proved to be stable, positive types and behaved like the wild types in further culture. About two thirds of the trans-

formed clones are unstable and more attention has been given to these types. Even after repeated purification the "segregating" clones throw off galactose-negative progeny at a rate of about one per 1,000 cell divisions. This rate is, however, variable from clone to clone and some so-called stable clones may be misclassified. The study of the unstable positive clones obtained from the combination of heterologous lysates and recipient cells justifies the following conclusions: (a) The unstable positive clones are heterozygous for the recipient and donor *Gal* markers but not for any other marker that has been tested. The term heterogenote is proposed for this type of partial diploid, which carries a transduced fragment or exogenote in addition to the basic genome. This structural interpretation is indicated by the reappearance among the segregants of the two specific *Gal*⁻ types which had entered into the transduction. In addition to the parental combinations, recombinations are also observed among the segregants. The recombinations include both the double *Gal*⁻ and the double *Gal*⁺, or wild type. (b) Most of the segregants appear to be haploid but about 6% are homogenotic for one of the *Gal* mutant markers. A consideration of these derived homogenotes shows that recombination can take place at a more than two-strand stage. (c) The most frequent type of segregant corresponds to the marker originally carried by the transductional recipient. The next most frequent type of segregant carries the *Gal* marker of the transductional donor and the least frequent type is a recombinant with respect to the two sets of markers.

4. Transductional properties of heterogenotic clones. As previously indicated, induced lambda from haploid *Lp*⁺ cultures has a low frequency of transduction, about 10⁻⁶ per phage particle. Induced lambda from heterogenotic clones has a much higher frequency of transduction approaching one per phage particle. Differential assays of these lysates indicate that the exogenote is preferentially included in the phage.

5. Phenotypic properties of heterogenotic clones; position effect. The occurrence of galactose-positive heterogenotes from the transductions of *Gal*⁺ to each of the *Gal*⁻ markers indicates that the *Gal*⁺ allele is dominant. In addition, all of the *cis* double heterogenotes of the form - -/_{ex} + + have been galactose-positive, as have been many but not all of the *trans* + -/_{ex} - + combinations. Among the types summarized in this paper, the particular combination *I*⁻⁴⁺/_{ex} *I*⁺4⁻ has proved to be galactose-negative, as has the reciprocal *I*⁺4⁻/_{ex} *I*⁻⁴⁺. The contrast between the phenotype of the two arrangements in this case thus indicates a *cis-trans* position effect. The structures of the *cis* and *trans* heterogenotes have been inferred both from their mode of formation and from their segregational pattern. The *Gal*₁, *Gal*₂, and *Gal*₄ markers have been most thoroughly studied from this point of view: in this group *Gal*₁ and *Gal*₄ show the positional interaction while none of the other combinations do so. Further studies involving the other *Gal*⁻ markers are in progress.

The first stage of transduction is the disruption of the donor genotype. In the DNA-transductions, this is accomplished when the cells are dissolved in bile salts; in phage-mediated transductions, it is an incident of phage growth. In *Salmonella* and the pneumococcus, any marker is readily transducible; the inclusion of exogenotes in *Salmonella* phage particles may be purely adventitious. In the lambda system, however, two restrictions are noted; 1) the phage, to be effective, must be prepared by the "induction" of a lysogenic bacterium (*Lp*⁺), and 2) the only factors that can be

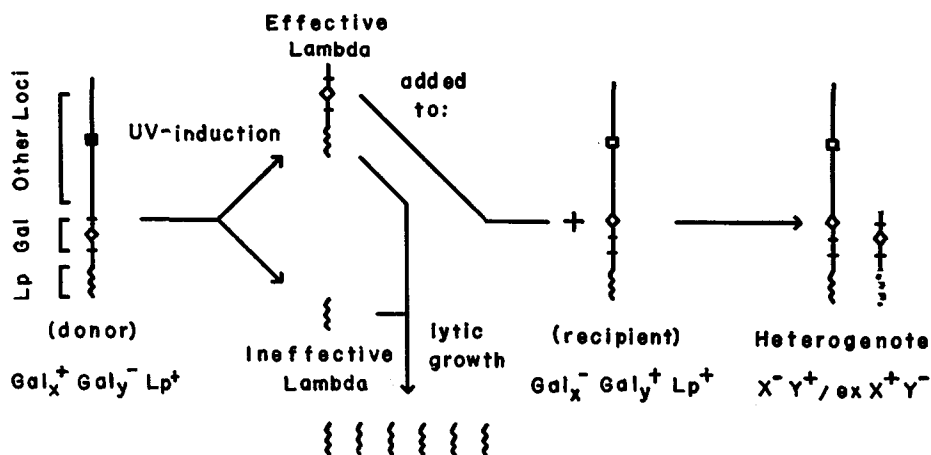


FIGURE 2.—Diagrammatic representation of the lambda-*E. coli* transduction system. The fate of the prophage (*Lp*) segment has not been worked out: this is indicated by the broken outline in the diagram of the heterogenote and by the omission of this segment from figures 3 and 4.

transduced are the *Gal* markers. Since it is precisely these markers which are linked to *Lp* (in crosses, LEDERBERG and LEDERBERG 1953; in transduction, LENNOX 1955 and JACOB 1955), we conclude that the effective exogenotes have a special relationship to lambda prophage. The simplest view of this relationship is diagrammed in figure 2, that the *Gal* markers and the prophage (*Lp*⁺) are adjacent segments of the bacterial chromosome, and that an effective particle is one in which induction has accidentally released a fragment which includes the *Gal* region, as well as the unique *Lp* segment. From this point of view, lysogenization, or the transfer of the prophage is itself a form of transduction, in which the behavior of *Lp* (prophage) is analogous to that of *Gal*. The unique features of the *Lp* segment which relate it to virus are the capacity for autonomous growth of *Lp*⁺ in the lytic cycle, and in "inducibility", i.e., the formation of specific coats in the maturation of phage when *Lp*⁺ bacteria are exposed to UV.

The envelopment of the *Gal-Lp* segment in the coat of a mature phage particle protects this material during its extracellular existence, and provides the means for its reentry into a new bacterium (assuming an analogy of lambda with *T*₂, HERSHEY and CHASE 1952). How these ends are accomplished with free DNA is known only on a complex, empirical basis.

At this point of our narrative, the exogenote has penetrated the recipient bacterium. This bacterium may be lysogenic, immune, or sensitive. Undoubtedly a fraction of transduction clones that would otherwise be detected from sensitive recipients are lost by lysis; how it is determined whether an infected bacterium will be lysed, lysogenized, or otherwise protected, is unknown.

The criterion for effectiveness in transduction is the formation of a galactose-positive clone, and therefore for the functional and reproductive integrity of the *Gal* segment. Ineffective particles may have exogenotes that were defectively prepared, or being intact, are improperly established in the recipient cell. The high activity of

HFT lysates shows that establishment is not a general limiting factor; the LFT lysates may be assumed to contain a heterogeneous population of particles, of which only a few contain an effective exogenote. The HFT quality of the lysates from syngenotes may be explained on the basis of selection of effective exogenotes, which had been screened by a prior selection for the ability to form a persistent galactose-positive heterogenote. The induction of a syngenote evidently preserves the integrity of the exogenote, as shown by its preferential inclusion in the phage yield, and perhaps by the reduced yield of phage from such a cell.

Two types of transformed clones have already been noted, stable and segregating. The stable clones evidently result from an early exchange of the exogenote with the recipient, leading to a reduced haploid product. The mechanism of this exchange cannot be readily studied (LEDERBERG 1955), but may be compared to the crossing over and reduction observed with persistent heterogenotes. However, the incidence of primary stable transformed clones (about $\frac{1}{3}$ of the total) is much higher than would be expected from the known rate of segregation in heterogenotic clones (10^{-3} per bacterial division, MORSE *et al.* 1956). It has also been observed that stable transduction clones are much less frequent in HFT transductions. The discrepancy might be explained either by postulating that the initial heterogenote is inherently unstable, or that LFT lysates include a moiety of particles that are capable of effective exchange, but not of initiating a persistent heterogenote. No data are yet available on the distribution of fragment sizes; the exogenotes so far studied (in HFT lysates from syngenotes) appear to encompass all the *Gal* markers.

More information about the mechanism of exchange can be obtained from the study of persistent heterogenotes. The three basic modes of division are diagrammed in figure 3. Most often, the heterogenote is propagated as such (*mitosis*); it may be *reduced* to a haploid, with or without concomitant crossing over; it may undergo internal recombination or *automixis*, i.e., engender a new syngenote of different constitution, for which one or more crossovers are implied. The types of automictic syngenotes already imply that crossing over occurs at a stage at which either the endogenote or the exogenote (presumably both) is already duplicated, i.e., at a "four-strand" stage. Proof that crossing over in a specific instance involves all four strands would entail, for example, the identification of a $++/_{ex}++$ homogenote from a $+ -/_{ex} - +$ heterogenote. Suspected occurrences of such types have not been completely analyzed (table 10).

Without more explicit information on centromere relationships, it is difficult to decide whether automixis is meiotic (reductive separation of centromeres) or mitotic (equational separation). Since a diploid *homozygote* has not been found as a product of automixis, the latter is preferred (see figure 3 for some of the postulated expectations). The term "somatic segregation" has been employed for analogous situations, but the issue here is not whether segregation occurs in a germinal or a somatic cell (whatever this would mean in the present context) but the underlying chromosome mechanics (STERN 1936).

The relationship of reduction to automixis is obscure. They may be concurrent, or as in *Aspergillus nidulans* (PONTECORVO, GLOOR, and FORBES 1954) the two processes may be independent, reduced crossover haploids being derived from syngenotes that

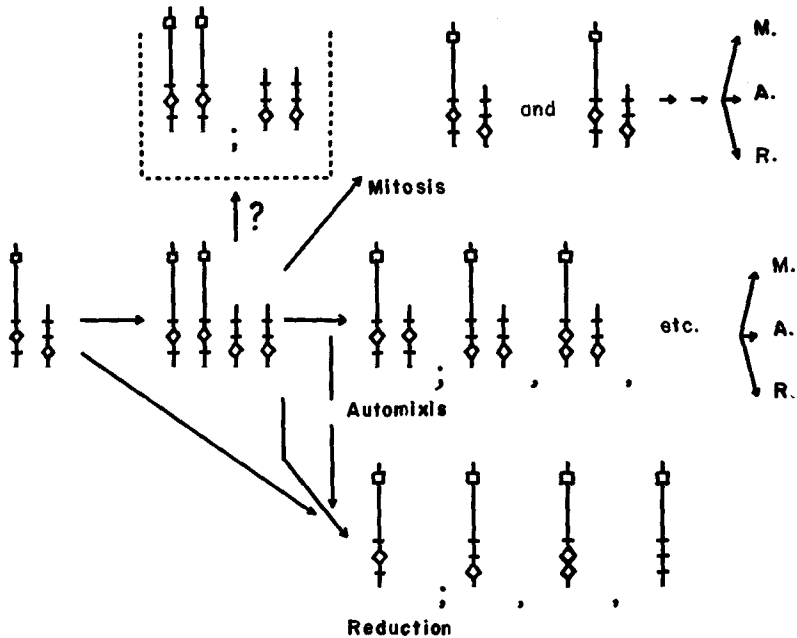


FIGURE 3.—Diagrammatic representation of modes of cell division occurring in syngenetic clones M, mitosis; A, automixis; R, reduction.

had undergone prior automixis. On this notion, reduction would result directly from the loss, nonreplication or nondisjunction of the exogenote. The prevalence of the endogenotic class of segregant is consistent with this view, but with most other formulations as well. This question may require a detailed pedigree analysis of a segregating clone before a definite answer is possible. Attempts to induce segregation of heterogenotes, e.g., with UV (cf. effects on heterozygotes, LEDERBERG *et al.* 1951) have been unsuccessful.

In figure 3, the exogenote is depicted as synapsed with the homologous endogenote. Specific synapsis must occur, at least to account for automictic events, but this is only one of several possibilities. On the one hand, the exogenote might be firmly attached to the endogenote, e.g., at the *Lp* site, to form a short "branch"; on the other it might lie free in the cell during vegetative growth.

The stability of heterogenotes in vegetative growth can be explained by assuming either 1) a definite association, synapsis or attachment, of the two elements throughout the division cycle or 2) regular equational disjunction of an independent exogenote which would behave, in effect, as a separate chromosome. Automictic events would necessitate at least an occasional synapsis even on the second view.

Whether it is transient or persistent, synapsis is a crucial mid-step of the transduction process. Transduction might be more prevalent than is now recognized, in the sense that exogenotes are transferred, but unless they retain the capacity for specific synapsis, they may be unable to attain a functional relationship with the cell. For example, the failure of lambda generally to mediate transduction of loci

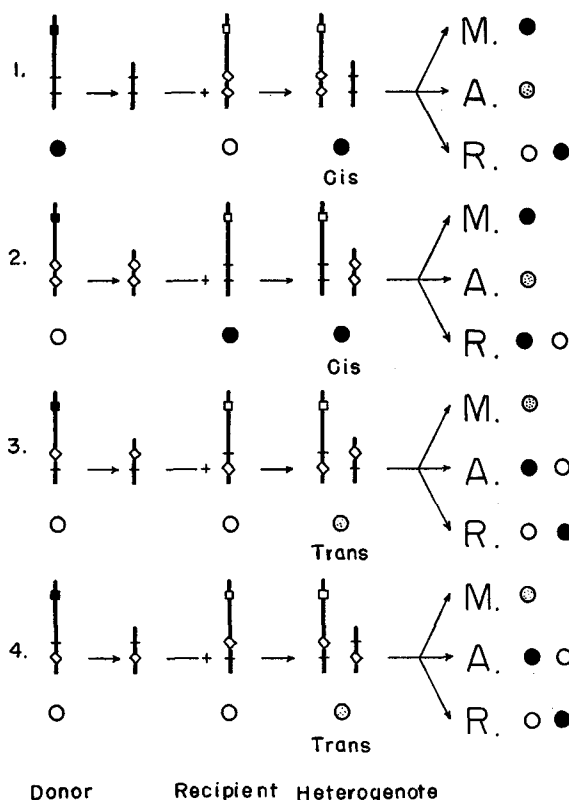


FIGURE 4.—The $GalH^-$, $GalK^-$ interactions (position effect). The structural formulae, inferred from the origins of the clones and from subsequent segregational behavior, are shown diagrammatically. The phenotypes are: ●, galactose positive; dotted ○, galactose negative, papillating; ○, galactose negative.

remote from *Lp* might be explained in these terms, rather than by their failure to be enveloped in the lambda particle. To put the burden of specific synapsis on the *Lp* segment is a plausible extrapolation from the regularity with which lambda combines with an *Lp*⁺ cell to form a lysogenic *Lp*⁺. However, other phages such as P1 do mediate generalized transduction in *E. coli* K-12. P1 and lambda might differ either in the quality of the nuclear fragments that are produced during viral growth (in regard to any critical step of transduction) or to the ability of the maturing phage coats to discriminate between prophage particles and other residues of the bacterial nucleus.

The uniqueness of each of the seven Gal^- markers that has been studied here parallels previous studies of the complexity of genetic loci. The uncovering of this complexity depends on the adequacy of recombination tests. In maize or *Drosophila*, 100,000 or a million tests would be considered exhaustive. The procedures described in this paper (HFT transduction and HFR crossing) could be extended to 10^9 or 10^{10} with comparable effort (compare crossing in maize, 10^5 , LAUGHAN 1955; LFT

transduction, 10^6 , DEMEREC *et al.* 1955; recombination in phage, 10^6 – 10^8 , BENZER 1955). The numerical comparisons should not be taken too seriously, as the most serious limitation is not the efficiency of the screening methods, but the interference from other factors, especially spontaneous mutation. Microorganisms have recently been the most prolific source of "pseudoallelism" but there is no reason to suppose that other organisms differ more profoundly than in limitations of technique.

Previous studies on bacteria have shown the prevalence of pseudoallelism and position effect for other loci in *E. coli* K-12. For example, heterozygotes of the *trans* type $Lac_{ia}^- Lac_{ib}^+ / Lac_{ia}^+ Lac_{ib}^-$ proved to be lactose-negative, while both single heterozygotes were lactose-positive (E. LEDERBERG 1952). Unfortunately, the techniques then available did not allow the positive identification of the *cis* heterozygote, $Lac_{ia}^+ Lac_{ib}^+ / Lac_{ia}^- Lac_{ib}^-$, which was assumed to be lactose-positive. In the *Gal* heterozygotes, the *cis* and *trans* arrangements have been compared directly (fig. 4), but they are yet to be studied in heterozygous diploids.

SUMMARY

The transduction of genetic material between cells of *E. coli* by phage lambda has been studied further. The material that could be transduced is limited to markers for the fermentation of galactose. Most of the transductional clones resulting carry a chromosomal fragment and are diploid for the genes which had been transduced. Such partially diploid clones, which have been named *heterogenotes*, segregate about once per thousand cell divisions, and give HFT⁺ lysates after UV induction. With the exception of the gene combinations, $Gal_1^- Gal_4^+ / Gal_1^+ Gal_4^-$, heterogenotes of the form $+/-$, $+ -/- +$, $- -/+ +$, etc., were galactose positive, indicating that the $+$ alleles are dominant. In the exceptional cases, *trans* combinations ($+ -/- +$) had a negative, the *cis* ($- -/+ +$) a positive phenotype, indicating a position effect between these loci. The heterogenotes undergo three modes of division: (1) mitosis, to propagate the original heterogenote; (2) reduction, to form haploid clones with markers of either the original recipient cell, transduced fragment, or recombinants of the two; (3) automixis, to form a new heterogenotic clone. This mode is analogous to somatic or mitotic crossing over.

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